Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

1
P69C
Cop. 2

LIBRARY
RECEIVED
MAY 1 [illegible] 1914
U. S. Department of Agriculture

Issued March 30, 1911.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—Circular No. 76.
B. T. GALLOWAY, Chief of Bureau.

THE RELATION OF CROWN-GALL TO LEGUME INOCULATION.

BY

KARL F. KELLERMAN,
Physiologist in Charge of Soil-Bacteriology and Water-Purification Investigations.

85091°—Cir. 76—11

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1911

BUREAU OF PLANT INDUSTRY.

Chief of Bureau, Beverly T. Galloway.
Assistant Chief of Bureau, William A. Taylor.
Editor, J. E. Rockwell.
Chief Clerk, James E. Jones.

B. P. I.—660.

THE RELATION OF CROWN-GALL TO LEGUME INOCULATION.

INTRODUCTION.

The relative merits of the inoculation of legumes by the pure-culture method and by the scattering of soil taken from old well-inoculated fields have been widely discussed, and both methods have been recommended by this Bureau. In case old soil is used, the fields from which it is secured should be free from objectionable weeds and insect pests and free from plant diseases.

From time to time specimens of supposedly well-inoculated plants of alfalfa, crimson clover, and alsike clover have been forwarded to the Laboratory of Soil-Bacteriology Investigations with the explanation that although nodules were produced in abundance the leguminous crop was not satisfactory. In these cases the appearance of the nodules was abnormal and the bacteria isolated from them, although resembling the nodule-forming organism, did not have the power of fixing nitrogen in culture solutions, and as a tentative explanation it was suggested[1] that this was but an extreme case of pleomorphism of *Pseudomonas radicicola*, which could be of no symbiotic advantage to the leguminous host.

During 1909 the organism occurring in the abnormal nodules of alfalfa was studied more extensively, and it was decided that this organism represented a new, although not a destructive, disease of alfalfa. Through the courtesy of Drs. Smith and Townsend a comparative study was made of material furnished by the Laboratory of Soil-Bacteriology Investigations, which showed that the bacteria causing the abnormal nodules upon alfalfa were practically identical with those causing the crown-gall of orchard trees.

In a recent bulletin[2] Smith, Brown, and Townsend have reported extensive studies upon the crown-gall of fruit trees, proving conclusively that this is not only an infectious disease caused by a specific micro-organism (*Bacterium tumefaciens*), but that it can infect many kinds of plants. For instance, the bacteria isolated from the crown galls of peach trees will produce excrescences upon the stems or upon the roots of tomatoes, sugar beets, salsify, rose bushes, apple trees, certain of the legumes, etc. The same is true of bacteria isolated from these abnormal galls, or tumors, upon sugar beets, and, in fact, it is probable that the crown-gall bacteria isolated from any one of the kinds of plants showing the disease will produce similar diseases in any of the other kinds of plants which have been found infected.

---

[1] Moore, George T. Soil Inoculation for Legumes; with Reports upon the Successful Use of Artificial Cultures by Practical Farmers. Bulletin 71, Bureau of Plant Industry, 1905.

[2] Smith, Erwin F., Brown, Nellie A., and Townsend, C. O. Crown-Gall of Plants; Its Cause and Remedy. Bulletin 213, Bureau of Plant Industry, 1911.

THE DISTINCTION BETWEEN CROWN-GALL TUMORS AND NITROGEN-FIXING NODULES.

Fortunately, the difference between nodules produced by the beneficial nodule-forming organism of the legumes and those produced by the crown-gall organism is sufficiently typical to be easily recognized by an experienced observer. If it is desired to use soil for inoculating new fields, an examination of the roots of the legume growing upon the old field would readily show whether the soil is suitable for distribution. The slight external difference between the crown-gall tumor and the nitrogen-fixing nodule is shown in Plate I, in which figure 1 shows the nitrogen-fixing nodules of alfalfa; figure 2, the crown-gall tumors; figure 3, the nitrogen-fixing nodules of crimson clover; figure 4, the crown-gall tumors. Though it may be possible to confuse these during a hasty examination, it is obvious upon close inspection that the nitrogen-fixing nodule is an outgrowth from the plant root, and that it has no more apparent effect upon the root than has an ordinary branch of the root. The interior of the nodule contains flesh-colored cells full of bacteria, which may be easily seen under the microscope. The crown-gall tumor, on the other hand, causes much distortion of the root, frequently forcing it to branch into many small roots, which project from the tumor itself. The interior of the tumor is white and it is difficult, if not impossible, to see any bacteria in any of the cells, even in the most carefully prepared sections of the tumor tissue.

With the facilities of a bacteriological laboratory it is not especially difficult to determine whether cultures distributed for inoculating legumes contain the crown-gall bacteria or the nodule-producing bacteria. The three tests most useful for a rapid diagnosis are as follows: Pour petri-dish plates with special colored agar.[1] The nodule-forming organism does not absorb the color, and grows as

---

[1] Water, 1,000 cubic centimeters; sugar, 10 grams; potassium phosphate (monobasic), 1 gram; magnesium sulphate, 0.2 gram; agar, 15 grams; and congo red, 0.1 gram.

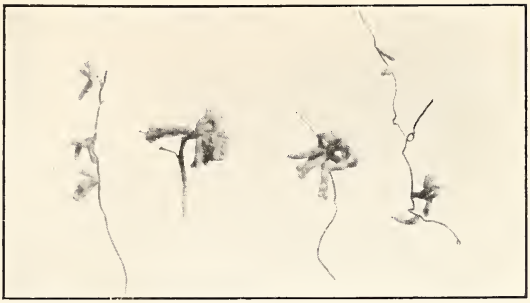

FIG. 1.—NITROGEN-FIXING NODULES OF ALFALFA.

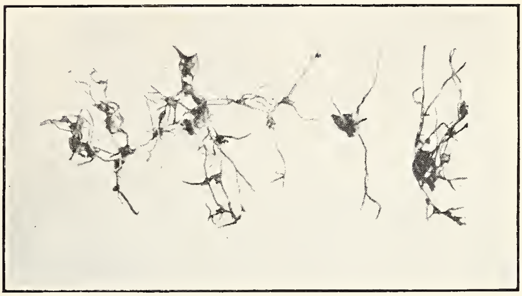

FIG. 2.—CROWN-GALL TUMORS OF ALFALFA.

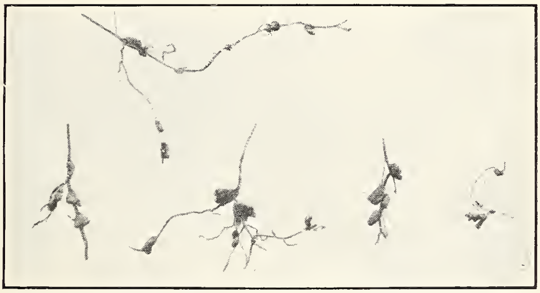

FIG. 3.—NITROGEN-FIXING NODULES OF CRIMSON CLOVER.

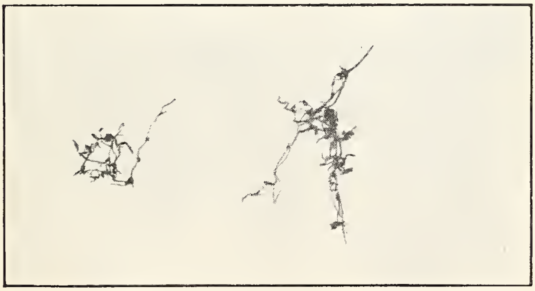

FIG. 4.—CROWN-GALL TUMORS OF CRIMSON CLOVER.

NITROGEN-FIXING NODULES AND CROWN-GALL TUMORS.

(Natural size.)

a whitish, semitranslucent, wet, shining colony. The crown-gall organism frequently absorbs the stain very freely and produces an intense red colony; even when not strongly colored the colonies are usually tinged with red, at least at the center. The clear whitish, shining colonies should be inoculated into Dunham's solution containing 2 per cent of nitrate and into a special synthetic broth.[1] After incubation for 10 days at 25° C., or 2 days at 37° C., the crown-gall organism from alfalfa produces sufficient nitrite to give an intense nitrite reaction with Griess's solution; the nodule organism produces no such reaction. In the synthetic broth the nodule organism reduces the nitrate sufficiently to give an appreciable nitrite reaction, and the alfalfa crown-gall organism does not.

CROWN-GALL INFECTION THROUGH LEGUMINOUS CROPS.

The fact that must be emphasized especially in connection with farm practice is that the excrescences, or tumors, formed on certain legumes by the crown-gall organism have occasionally been confused with the desirable nitrogen-fixing nodules. It is obvious, therefore, that in all orchard or sugar-beet regions the possible danger of crown-gall infection through leguminous crops must be considered. The use of soil for inoculating alfalfa or clover, if selected at random, may be a serious menace. In the few years that this matter has been under observation many records of the shipping of alfalfa soil infected with crown-gall under the designation of inoculated alfalfa soil have been obtained.

It is not impossible that cultures prepared by bacteriologists unfamiliar with the slight, though characteristic, physiological differences between the nodule organism and the crown-gall organism might serve as a means of infecting a clean field planted with culture-treated seed. As it is the custom of the bacteriologists of the agricultural experiment stations, and also of the bacteriologists connected with commercial firms, to isolate cultures only from well-grown normal nodules, it is not probable that this second possible source of crown-gall infection is important.

Whether a leguminous crop infected with crown-gall constitutes an unusually serious menace to succeeding crops of sugar beets or to orchards has not yet been determined. The three leguminous crops, alfalfa, crimson clover, and alsike clover, seem to serve as very favorable hosts for *Bacterium tumefaciens*, and the organism isolated from these legumes appears to be at least as viable as when isolated from its other hosts. It is well known that most leguminous crops greatly magnify the severity of nematode infection for succeeding crops in regions where this pest occurs, and this analogy emphasizes the necessity, at least until the lack of the necessity for such caution has been demonstrated, of using the utmost care in guarding against the dissemination and the favoring of crown-gall through the agency of these crops.

---

[1] Water, 1,000 cubic centimeters; sugar, 10 grams; potassium phosphate (monobasic), 1 gram; magnesium sulphate, 0.2 gram, and 0.2 per cent of potassium nitrate.

LOCALITIES WHERE CROWN-GALL HAS BEEN FOUND.

At this time we have records of the occurrence of crown-gall tumors upon alfalfa in central Kentucky, southwestern Alabama, northern Virginia, southern Maryland, and northern Pennsylvania.

In greenhouse plants grown at Washington, D. C., in infected soil received from Kentucky, alsike clover, crimson clover, red clover, and alfalfa showed extensive infection, and it is probable that these plants will be found naturally infected in different parts of the United States.

CONCLUSIONS.

(1) The crown-gall organism has been found in tumors somewhat resembling the normal nitrogen-fixing nodules upon the roots of alfalfa, crimson clover, and alsike clover.

(2) Great care should be taken in using soil or cultures for inoculating legumes in regions which may eventually be used for sugar beets or for orchards.

(3) It is usually possible to distinguish the tumor produced by the crown-gall bacteria from the nodule formed by the nitrogen-fixing bacteria by their external appearance.

(4) By the use of special media it is possible to distinguish between the bacterium which causes crown-gall and the nitrogen-fixing bacterium which forms the desired nodules upon the roots of leguminous plants.

(5) It is not known what other leguminous crops are susceptible to crown-gall infection. It is believed, however, that there is reason to suspect all of the clovers.

Approved:
JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, D. C., *March 17, 1911.*